Original article

MOLECULAR METABOLISM

Therapeutic effects of adropin on glucose tolerance and substrate utilization in diet-induced obese mice with insulin resistance

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ABSTRACT

Objective: The peptide hormone adropin regulates fuel selection preferences in skeletal muscle under fed and fasted conditions. Here, we investigated whether adropin treatment can ameliorate the dysregulation of fuel substrate metabolism, and improve aspects of glucose homeostasis in diet-induced obesity (DIO) with insulin resistance.

Methods: DIO C57BL/6 mice maintained on a 60% kcal fat diet received five intraperitoneal (i.p.) injections of the bioactive peptide adropin$^{34-76}$ (450 nmol/kg/i.p.). Following treatment, glucose tolerance and whole body insulin sensitivity were assessed and indirect calorimetry was employed to analyze whole body substrate oxidation preferences. Biochemical assays performed in skeletal muscle samples analyzed insulin signaling action and substrate oxidation.

Results: Adropin treatment improved glucose tolerance, enhanced insulin action and augmented metabolic flexibility towards glucose utilization. In muscle, adropin treatment increased insulin-induced Akt phosphorylation and cell-surface expression of GLUT4 suggesting sensitization of insulin signaling pathways. Reduced incomplete fatty acid oxidation and increased CoA/acetyl-CoA ratio suggested improved mitochondrial function. The underlying mechanisms appear to involve suppressions of carnitine palmitoyltransferase-1B (CPT-1B) and CD36, two key enzymes in fatty acid utilization. Adropin treatment activated pyruvate dehydrogenase (PDH), a rate-limiting enzyme in glucose oxidation, and down-regulated PDH kinase-4 (PDK-4) that inhibits PDH. Along with these changes, adropin treatment downregulated peroxisome proliferator-activated receptor-gamma coactivator-1α that regulates expression of *Cpt1b, Cd36* and *Pdk4*.

Conclusions: Adropin treatment of DIO mice enhances glucose tolerance, ameliorates insulin resistance and promotes preferential use of carbohydrate over fat in fuel selection. Skeletal muscle is a key organ in mediating adropin's whole-body effects, sensitizing insulin signaling pathways and altering fuel selection preference to favor glucose while suppressing fat oxidation.

Keywords Adropin; Glucose metabolism; Fatty acid metabolism; Insulin action; Metabolic flexibility; Mitochondrial function

1. INTRODUCTION

Adropin is a small peptide that has been linked to metabolic homeostasis and cardiovascular function [1–4]. High levels of expression of the *Energy Homeostasis* Associated (*Enho*) gene encoding adropin have been observed in the central nervous system, although widespread expressions in peripheral tissues such liver, cardiac and skeletal muscle, and endothelium have also been reported [4–6]. Adropin was originally proposed to be a secreted factor, with residues 1-33 encoding a secretory signal peptide [4]. A more recent study suggests that adropin might be a membrane-bound protein that interacts with the notch signaling pathway to modulate intercellular communications [5]. While the source and mechanism of release remains controversial, adropin immunoreactivity has nevertheless been reported by several laboratories to be present in plasma and sera of mouse, nonhuman primate and human [3,7–23]. Studies in mice suggest that the gene expression and the circulating levels of adropin are affected by dietary macronutrients and energy balance states [3,4,20,24,25].

The rapid regulation of adropin levels by nutritional and energy states points to potential roles for adropin in metabolic homeostasis. Indeed, early studies showed that transgenic overexpression of adropin or treatment using the putative secreted domain (adropin$^{34-76}$) improved glucose clearance, reduced fasting insulin and reversed dyslipidemia and the fatty liver phenotype in diet-induced obese C57BL/6 (DIO) mice [4]. In addition, our group observed evidence of insulin resistance in

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the adropin knockout mice [20]. Furthermore, we recently proposed that adropin regulates the preference for fuel selection in skeletal muscle in the feeding and fasting cycle [3]. We posited that increased release of adropin, such as in the fed state, activates pyruvate dehydrogenase (PDH) complex to increase glucose oxidation [3]. In parallel, adropin reduces muscle fatty acid oxidation (FAO) by inhibiting carnitine palmitoyltransferase-1B (CPT1B) [3], a key enzyme that transports fatty acids into muscle mitochondria for β-oxidation [26].

Dysregulation of glucose and fatty acid metabolism is a metabolic signature in the diet-induced obese (DIO) state [27]. In the DIO condition, glucose utilization is diminished and fatty acids are the predominant fuel source in muscle [27]. One mechanism explaining altered fuel selection preference involves the excessive FAO that inhibits pyruvate and glucose oxidation by the Randle cycle mechanism [28–31]. A growing body of evidence suggests that limiting excessive FAO in muscle plays a role in maintaining glucose homeostasis in DIO rodents [30–32]. As our data suggest that adropin is a physiological regulator of the oxidation of glucose and fatty acid, we speculated that adropin treatment would exert therapeutic roles in ameliorating the dysregulated fuel metabolism and glucose intolerance in DIO state. Indeed, recent evidence indicates that high-fat feeding results in muscle mitochondrial fatty acid overload and excessive β-oxidation, which has been proposed to contribute to the development of insulin resistance in DIO mice [30]. Furthermore, it has been suggested that inhibition of muscle FAO can alleviate insulin resistance in DIO mice [32]. Taking these observations together, we hypothesized that adropin treatment would enhance insulin actions in muscle of DIO mice. The current study investigated whether adropin treatment would impact substrate utilization, improve glucose homeostasis and ameliorate insulin resistance in the diet-induced obesity.

2. MATERIALS AND METHODS

2.1. Animal studies

Mouse experiments were approved by the Institutional Animal Care and Use Committees of the Scripps Research Institute (Jupiter, Florida). Male, lean or DIO C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Lean mice were maintained on low-fat diets (10–14% kcal fat). DIO mice were maintained on high fat diet (60% kcal fat) (Research Diets, New Brunswick, NJ). Mice were monitored daily after shipment until body weight became stabilized. Mice subjected to experimental procedures were 24-week old. Body composition was determined using a NMR spectroscopy analyzer (Bruker Minispec).

2.2. Adropin treatment

Adropin$^{34-76}$ was provided by Ipsen (Paris, France) or purchased from ChinaPeptides (Shanghai, China). The peptide was dissolved in 0.1% bovine serum albumin, and administered by intraperitoneal (i.p.) injection.

2.3. Glucose and insulin tolerance tests

The animal handling and injection protocols used for glucose tolerance test (GTT) (glucose, 2 mg/g fat-free mass) and insulin tolerance test (ITT) (Humulin, Eli Lilly, IN; insulin, 0.5 mU/g body weight) are shown in Figure 1. Blood glucose levels were monitored using OneTouch Blood Glucose Meters (LifeScan Europe, Switzerland) at the times indicated. Serum insulin levels were measured using an Ultrasensitive Mouse Insulin ELISA kit (Crystal Chem, Downers Grove, IL).

2.4. Whole body metabolic assessment

Oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER; VCO_2/VO_2) were measured using a

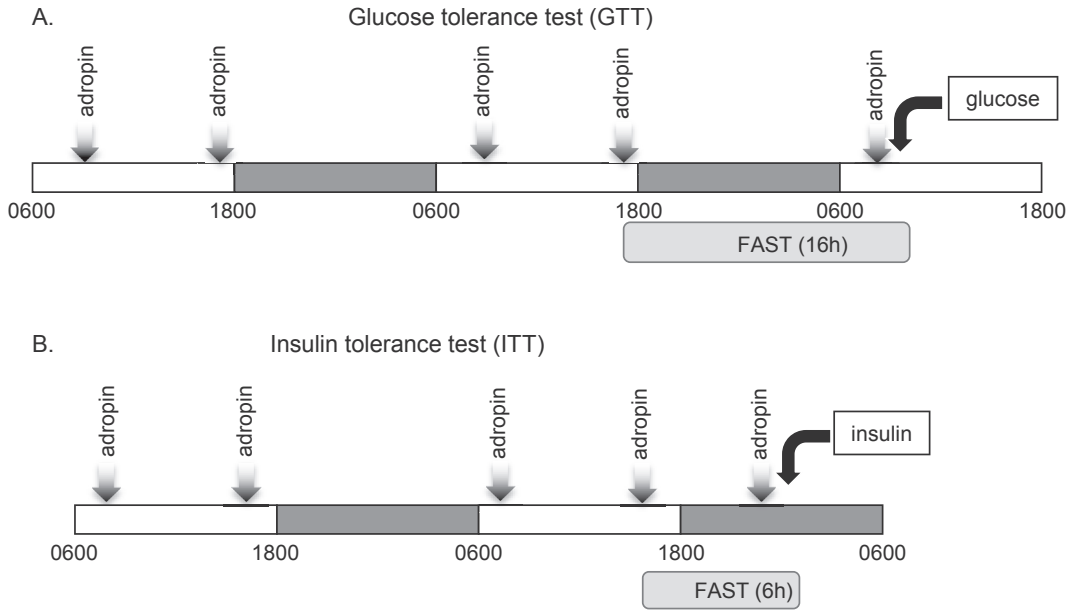

Figure 1: Schemes of the animal treatment for the assessments of glucose and insulin tolerance in DIO mice. DIO mice received five intraperitoneal (i.p.) injections of adropin$^{34-76}$ or vehicle over a 48 h period. A group of chow-fed lean mice included in the study received injections of vehicle. (A) Protocol for assessing the impact of adropin treatment on glucose tolerance. After the 4th injection of adropin or vehicle, food was removed and the mice fasted overnight. The mice received a 5th injection the next morning; one hour later, baseline blood glucose levels were determined (t = 0); mice then received an i.p. injection of glucose (2 mg/g fat free mass). Glucose levels were then determined at 15 min intervals. (B) Protocol for assessing the impact of adropin treatment on insulin tolerance. DIO and the lean control mice received five intraperitoneal (i.p.) injections of adropin$^{34-76}$ or vehicle. One hour after the 5th injection, the mice that had been fasted for 6 h were given an i.p. injection of insulin (0.5 mU/g body weight).

